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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Danishefsky *et al.* Examiner: T. Solola
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For: *Synthesis of Epothilones, Intermediates Thereto, Analogues and Uses Thereof*

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Paragraph and Section Replacements for Preliminary Amendment

A) A replacement paragraph is provided below, which paragraph begins on page 1, line 27 and ends on page 1, line 34.

This application is a continuation application filed under 37 C.F.R. § 1.53(b) of application number 08/986,025, filed December 3, 1997, now U.S. PAT. NO. 6,242,469, the entire contents of which are hereby incorporated by reference, which is based on U.S. Provisional Application Serial Nos. 60/032,282, 60/033,767, 60/047,566, 60/047,941, and 60/055,533, filed December 3, 1996, January 14, 1997, May 22, 1997, May 29, 1997, and August 13, 1997, respectively, the contents of which are hereby incorporated by reference into this application. This invention was made with government support under grants CA-28824, CA-39821, CA-GM 72231, CA-62948, and AI0-9355 from the National Institutes of Health, and grant CHE-9504805 from the National Science Foundation. Additionally, the present invention was supported in part by a fellowship from the United States Army to Dongfang Meng (DAMD 17-97-1-7146), and thus the government has certain rights in the invention.

B) A replacement section is provided below, which section begins on page 3, line 21 and ends on page 3, line 22.

Figures 3(A) and 3(B) provide syntheses of key iodinated intermediates used to prepare

AA2 cont'd hydroxymethylene- and hydroxypropylene-substituted epothilone derivatives.

C) A replacement section is provided below, which section begins on page 3, line 24 and ends on page 3, line 27.

AA3 Figures 3(C) and 3(D) provide methods of preparing hydroxymethylene- and hydroxypropylene-substituted epothilone derivatives, said methods being useful generally to prepare 12,13-E epothilones wherein R is methyl, ethyl, n-propyl, and n-hexyl from the corresponding E-vinyl iodides.

D) A replacement section is provided below, which section begins on page 3, line 29 and ends on page 3, line 30.

AA4 Figures 3(E) and 3(F) show reactions leading to benzoylated hydroxymethyl-substituted desoxyepothilone and hydroxymethylene-substituted epothilone (epoxide).

E) A replacement section is provided below, which section begins on page 4, line 9 and ends on page 4, line 9.

AA5 Figures 6(A) and 6(B) provide a scheme of an olefin metathesis route to epothilone A and other analogues.

F) A replacement section is provided below, which section begins on page 4, line 29 and ends on page 4, line 29.

AA6 Figures 14(A) and 14(B) show the preparation of intermediate **4A**.

G) A replacement section is provided below, which section begins on page 5, line 7 and ends on page 5, line 8.

Figures 18(A) and 18(B) provide a synthetic pathway to a protected intermediate for 8-desmethyl deoxyepothilone A.

H) A replacement section is provided below, which section begins on page 5, line 10 and ends on page 5, line 11.

Figures 19(A), 19(B), and 19(C) provide a synthetic pathway to 8-desmethyl deoxyepothilone A and a trans-iodoolefin intermediate thereto.

I) A replacement section is provided below, which section begins on page 5, line 13 and ends on page 5, line 22.

Figure 20(A) shows structures of epothilones A and B and 8-desmethylepothilone and Figure 20(B) shows a synthetic pathway to intermediate TBS ester **10** used in the preparation of desmethylepothilone A. (a) (Z)-Crotyl-B[(-)-Ipc]$_2$, -78°C, Et_2O, then 3 N NaOH, 30% H_2O_2; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2 (74% for two steps, 87% ee); (c) O_3, CH_2Cl_2/MeOH, -78°C, then DMS, (82%); (d) t-butyl isobutyrylacetate, NaH, BuLi, 0°C, then **6** (60%, 10:1); (e) $Me_4NBH(OAc)_3$, -10°C (50%, 10:1 α/β) or $NaBH_4$, MeOH, THF, 0°C, (88%, 1:1 α/β); (f) TBSOTf, 2,6-lutidine, -40°C, (88%); (g) Dess-Martin periodinane, (90%); (h) $Pd(OH)_2$, H_2, EtOH (96%); (l) DMSO, oxalyl chloride; CH_2Cl_2, -78°C (78%); (j) Methyl triphenylphosphonium bromide, NaHMDS, THF, 0°C (85%); (k) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (87%).

J) A replacement section is provided below, which section begins on page 5, line 29 and ends on page 5, line 29.

Figures 22(A), 22(B) and 22(C) show a synthetic pathway to prepare epothilone analogue **27D**.

K) A replacement section is provided below, which section begins on page 5, line 31 and ends

on page 5, line 31.

Figures 23(A), 23(B) and 23(C) show a synthetic pathway to prepare epothilone analogue **24D**.

L) A replacement section is provided below, which section begins on page 5, line 33 and ends on page 5, line 33.

Figures 24(A) and 24(B) show a synthetic pathway to prepare epothilone analogue **19D**.

M) A replacement section is provided below, which section begins on page 5, line 35 and ends on page 5, line 35.

Figures 25(A), 25(B), 25(C) and 25(D) show a synthetic pathway to prepare epothilone analogue **20D**.

N) A replacement section is provided below, which section begins on page 5, line 37 and ends on page 5, line 37.

Figures 26(A), 26(B), 26(C) and 26(D) show a synthetic pathway to prepare epothilone analogue **22D**.

O) A replacement section is provided below, which section begins on page 6, line 1 and ends on page 6, line 2.

Figures 27(A), 27(B) and 27(C) show a synthetic pathway to prepare epothilone analogue 12-hydroxy ethyl epothilone.

P) A replacement section is provided below, which section begins on page 6, line 4 and ends on page 6, line 7.

Figures 28(A) and 28(B) show the activity of epothilone analogues in a sedimentation test in comparison with DMSO, epothilone A and/or B. Structures 17-20, 22, and 24-27 are shown in Figures 29-37, respectively. Compounds were added to tubulin (1 mg/ml) to a concentration of 10 μM. The quantity of microtubules formed with epothilone A was defined as 100%.

Q) A replacement section is provided below, which section begins on page 6, line 30 and ends on page 6, line 32.

Figures 39(A) and 39(B) show epothilone A and epothilone analogues #1-7. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

R) A replacement section is provided below, which section begins on page 6, line 34 and ends on page 6, line 36.

Figures 40(A) and 40(B) show epothilone B and epothilone analogues #8-16. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

S) A replacement section is provided below, which section begins on page 7, line 1 and ends on page 7, line 3.

Figures 41(A) and 41(B) show epothilone analogues #17-25. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

T) A replacement section is provided below, which section begins on page 7, line 5 and ends on page 7, line 7.

Figures 42(A) and 42(B) show epothilone analogues #26-34. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

U) A replacement section is provided below, which section begins on page 7, line 10 and ends on page 7, line 12.

Figures 42(C) and 42(D) show epothilone analogues #35-46. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

V) A replacement section is provided below, which section begins on page 7, line 14 and ends on page 7, line 14.

Figures 42(E) shows epothilone analogues #47-49.